Vår ref:2013/H_114
Deres ref: 2013/3791

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 16.09.13

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet om høringen **EFSA/GMO/NL2013/114 (2013/3791)** som gjelder søknad om bruk av genmodifisert bomull MON 88701.

Vennligst ta kontakt hvis du har noen spørsmål.

Med vennlig hilsen,

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Vår ref:2013/H_114
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Assessment of the technical dossier submitted under EFSA/GMO/NL2013/114 for approval of MON 88701 cotton from Monsanto Company

Submitted to

Miljødirektoratet

by

Rosa Binimelis, Idun Grønsberg, Lise Nordgård, Vinicius Vilperte

Centre for Biosafety – GenØk
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KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av genmodifisert bomull MON 88701.

Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til MON 88701 som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

Hovedkonklusjon og anbefalinger
Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående høring som omfatter MON 88701 for bruksområdet mat, fór, import og prosessering. Planten blir i følge søknaden tolerant mot plantevernmidlene dicamba og glufosinat-ammonium.

MON88701, er en stablet hybrid med ulike herbicid-toleranse-kodende gener innebygd. Stablede hybridplanter har generelt en mer kompleks genetisk sammensetning og derfor større potensiale for opp- og nedregulering av plantens egne gener. En grundig testing før evt markedsadgang vil derfor være nødvendig. Søker bør fremskaffe eksperimentelle bevis som viser at kombinasjonen ikke er skadelig og ikke bare vise til antagelser basert på vurderinger gjort av disse proteinene hver for seg.

CS-dmo-proteinet gjør bomulls planter tolerante overfor ugrasmidler med virkestoffet dicamba. Dicamba har vært betegnet som et plantevernmiddel med lav toksisitet. I den senere tid har det vært publisert artikler som indikerer indirekte negative effekter av dicamba på insekter. Søker bør derfor undersøke nærmere potensielle miljø- og helsemessige effekter.

I tillegg er plantevernmidlet glyfosat-ammonium ikke lovlig i Norge eller EU. Vi mener en godkjennelse av MON 88701vil skade grunnleggende etiske og sosiale kriterier for bruk, som omtalt i den norske Bioteknologiloven.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke vedlagt søknaden om omsetting av mat produsert fra MON 88701 eller inneholdende ingredienser produsert fra MON 88701

Vår konklusjon er at norske myndigheter på bakgrunn av slik søknaden foreligger i dag ikke godkjenner bruk av MON 88701 for bruksområdene mat, fór, import og prosessering som det søkes om.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL2013/114

As a designated National Competence Center for Biosafety, our mission at GenØk in advice giving is to provide independent, holistic and useful analysis of technical and scientific information/reasoning in order to assist authorities in the safety evaluation of biotechnologies proposed for use in the public sphere.

We have targeted our critique to the relevant provisions that relate to our particular area of competence in biotechnology assessment as comprehensively as possible. Lack of commentary on our part towards any information under consideration should not be interpreted as specific endorsement of that information.

The following information is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of MON 88701, setting out the risk of adverse effects on the environment, including other consequences of proposed release under the pertinent Norwegian regulations.

Specific recommendations

Based on our findings, we propose a few specific recommendations, summarized here and detailed in the critique below.

- The regulator is encouraged to ask the Applicant for a more detailed evaluation of how *S. maltophilia* causes diseases and if there are any known or plausible contribution to pathogenicity by DMO.
- The regulator is encouraged to ask the Applicant to provide direct evidence of the lack of combinatorial effects arising from the expression of the stacked proteins in the plant, instead of relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.
- The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides/pesticides on the same plant.
- The regulator is encouraged to ask the Applicant to address the potential influence of dicamba on food-web dynamics.
- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.
- The regulator is encouraged to ask the Applicant to considering recent scientific findings, and extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.
- The regulator is encouraged to ask the Applicant to provide additional data using a

comprehensive set of smaller probes in order to evaluate the genetic stability of the event.

- The regulator is encouraged to ask the Applicant to follow the same methodology for generational stability should as the others southern blot analysis (i.e. using the same probes).
- The regulator is encouraged to ask the Applicant to present molecular size markers when facilitating the analyses.

- The regulator is encouraged to ask the Applicant to use molecular profiling techniques to allow a more thorough study of the insert genetic stability over multiple generations.
- The regulator is encouraged to ask the Applicant to provide the electropherograms in order to check the quality of the sequences.
- The regulator is encouraged to ask the Applicant to conduct generational sequencing studies.
- The regulator is encouraged to ask the Applicant to clarify if the mode of treatment is as it would be performed during agricultural production.
- The regulator is encouraged to ask the Applicant to comment on the difference in the DMO and PAT expression levels in the treated and non-treated seed.
- The regulator is encouraged to ask the Applicant to clarify size determination and bands in the protein analysis and provide pictures that makes it possible to draw conclusions.
- The regulator is encouraged to ask the Applicant to consider toxicity study with the two proteins in combination.
- The regulator is encouraged to ask the Applicant to use the plant version of the protein in the animal feeding studies to obtain representative results.
- The regulator is encouraged to as the Applicant to include long term exposure-/feeding, using herbicide treated cotton in animal experiment, before a GM plant product is released on the marked for food/feed consumption.
- The regulator is encouraged to ask the Applicant to clarify at what pH the digestion studies are performed.
- The regulator is encouraged to ask the Applicant to perform serum screening for allergenicity purpose.
- The regulator is encouraged to ask the Applicant to submit required information on the social utility of MON 88701 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Overall recommendation

From our analysis, we find that the deficiencies in the dossier do not support claims of safe use, social utility and contribution to sustainable development of **MON 88701**. **Critically, the Applicant has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway.** Hence at minimum, the dossier is deficient in information required under Norwegian law. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of MON 88701, we conclude that based on the available data supplied by the Applicant, the Applicant has not substantiated claims of environmental safety satisfactorily or provide the required information under Norwegian law to warrant approval in Norway at this time.

ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL2013/114

About the event

The genetically modified MON 88701 cotton was generated through *Agrobacterium* mediated transformation. The genetic modification intended to be inserted was a *dmo* and a *bar* expression cassette (T-DNA). The presence of MON 88701 DMO protein confers dicamba tolerance and the presence of PAT (bar) protein confers glufosinate tolerance.

The Applicant is requesting the authorization for GM plants for food, feed, and import and processing.

Assessment

Stacked events

Innovation for agriculture moves towards more complex stacked transgene combinations, with multiple insect-toxins and tolerance to multiple herbicides/pesticides within the same plant. Stacked events are in general more complex and it has been an increased interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes in the plant – like the potential for up- and down regulation of the plants own genes. Interactions with stacked traits cannot be excluded that the group of expressed toxins in the plant can give specific immunological effects or adjuvant effects in mammals (Halpin 2005, Schrijver et al, 2007).
In addition, there is an increasing need to test potential environmental consequences of these technologies, and assess their risks, e.g. studies of combinatorial effects of multiple stressors that are increasingly acknowledged as missing, e.g. from EFSA (EFSA GMO Panel Working Group on Animal Feeding Trials, 2008).

Recommendation:

- The regulator is encouraged to ask the Applicant to provide direct evidence of the lack of combinatorial effects arising from the expression of the stacked proteins in the plant, instead of relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.
- The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides/pesticides on the same plant.

Herbicides

Dicamba

The genetically modified MON 88701 cotton expresses a *dmo* gene that confer tolerance to herbicide products containing dicamba. Dicamba is a benzoic acid herbicide that mimics the plant hormone auxin, causing uncontrolled growth which eventually kills plants.
Dicamba is considered as an herbicide with low toxicity, but with high residuality. In recent years dicamba has received more risk-related attention due to the on-going evolution of glyphosate resistance in weed species and use of other agrochemicals in some agroecosystems

(Binimelis et al 2009, Ensminger et al 2013). A recent article has been published indicating indirect negative effects of dicamba on insects, while highlighing that few research has been conducted on the issue to date in despite dicamba is, along with 2,4-D, causing most herbicide-drift damage to nontarget plants even though present limited agricultural usage (Love et al 2011, Bohnenblust et al., 2013).

Recommendation:

- The regulator is encouraged to ask the Applicant to address the potential influence of dicamba on food-web dynamics.
- The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides/pesticides on the same plant.

Glufosinate-ammonium

The *pat* gene derived from *Streptomyces viridochromogenes* confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Studies have shown that glufosinat ammonium is harmful by inhalation, swallowing and by skin contact and serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where glufosinate ammonium is used (Hung 2007, Matsumura et al. 2001, Schulte-Hermann et al. 2006, Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

Recommendation:

- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.

2 Molecular characterizations (p. 20)

2.1 Information relating to the genetic modification (p. 20)

Source of donor DNA
The Applicant uses a demethylase gene from *Stenotrophomonas matophilia* that expresses a dicamba mono-oxogenase (DMO) protein to confer tolerance to dicamba herbicide. The Applicant claims it safety except for the potential of *S. maltophilia* to cause infections in immunocompromised patiens (p. 31). However the Applicant does not mention the number of cases of infections the recent years, mortality of those with infection etc.

S. maltophilia is an environmental global emerging Gram-negative multiple-drug-resistant-organisms (MDRO) that is most commonly associated with respiratory infections in humans. The frequency of infections related to *S. maltophilia* has increased the last decade and the mortality rate in patients with bacteremia has also been reported to be quite high (Denton and Kerr, 1998, Brooke JS, 2012).

Recommendation:
- The regulator is encouraged to ask the Applicant for a more detailed evaluation of how *S. maltophilia* causes diseases and if there are any known or plausible contribution to pathogenicity by DMO.

2.2 Information relating to the GM plant (p. 37)

Information on the sequences actually inserted/deleted or altered (p.41)
The article from Podevin and Du Jardin (2012) has created a discussion related to if past approvals of GM events have overlooked key safety questions related to the use of the Cauliflower Mosaic Virus 35S promoter (P35S) in GM plants. In the article, the authors state that some P35S variants contain open reading frames that when expressed could lead to "unintended phenotypic changes. Gene VI encodes the multifunctional P6 protein that can be divided into four domains (Li and Leiser, 2002). Functions of P6 include nuclear targeting (Haas et al. 2008), viral particle binding and assembly (Himmelbach et al. 1996), si- and ds-RNA interference and interference suppression (Shivaprasad et al. 2008) and transcriptional transactivation (Kobayashi et al 2004; Palanichelvam et al. 2002).

Since the *bar* expression cassete is under the regulation of the e35S promoter and the *dmo* expression cassete is under the regulation of the *PC1SV* promoter (Peanut Chlorotic Streak Caulimovirus), which belongs to the same genus as the CaMV, the applicant should be required to study the presence of partial P6 protein and the possibility of chimeric proteins containing P6 fragments in these promoter sequences.

A study by Rang et al. (2005) revealed the possibility for read-through of the NOS terminator in GTS 40-3-2 soybean resulting in four different RNA variants with the potential to express unknown EPSPS fusion proteins. Since the *bar* expression cassete contains the NOS terminator, the applicant should study carefully the possibility of read-through resulting in different RNA variants and potential fusion proteins.

The sizes of most of the probes in the Southern Blot analyses are considered too long and they can lead to false negative results. A long probe that binds perfectly to a short insertion will not be strongly bound and may be washed off depending on the stringency of the wash. For example, figure 11, lanes 2 and 8 (p.54) showed weak band for the higher band. This occurred, probably, because of the long probe (1,1kb) binding in a small fragment.

All Southern Blot pictures are lacking the molecular weight marker, which is essential to confirm the sizes of the digested products. For instance, figure 9, lane 5 (p.52) shows a conventional sample spiked with probes 1 and 5. The higher band should be around 1,3kb, but, according to the arrow, the size is 1,6kb.

For the Generational Stability studies, five generations were used for the Southern blot studies. However, only two probes were used (which did not span the whole insert) and the samples were digested only with one restriction enzyme. The applicant should use the same approach used in the previous Southern Blot analyses.

The use of Southern Blot as the only method to examine the genetic stability of MON 88701 cotton is not advisable since they can only give information on the gross structure and copy number of the insert. Small rearrangements and small deletions as well as point mutations that might result in the formation of new ORFs or changes in the expressed protein will not be detected (De Shrijver et al. 2007). The use of molecular profiling techniques (Heinemann et al. 2011) is highly recommended.

In the sequence studies, the applicant states that *"As expected, a ~4.5 kb PCR product (Figure 11, lane 3) spanning the entire sequence between Primer A and Primer B in MON 88701 was not amplified in this analysis, because the PCR conditions necessary to generate a product of this size were not used"*. Additional data should be supplied showing the presence of the 4.5kb band under optimal conditions.

The Applicant also states that *"Sequencing electropherograms were rejected if they were of unacceptable quality, particularly with respect to peak shape and intensity. None of the rejected data was inconsistent with the conclusions presented in this report."* The electropherograms are not available; therefore it is not possible to evaluate the quality of the sequences.

The study was conducted only with plants from one generation. Since Southern Blot analyses for five generation were conducted and since this analysis is not able to detect small rearrangements, sequencing analysis should have been conducted as well.

Recommendations:

- The regulator is encouraged to ask the Applicant to considering recent scientific findings, and extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.
- The regulator is encouraged to ask the Applicant to provide additional data using a comprehensive set of smaller probes in order to evaluate the genetic stability of the event.
- The regulator is encouraged to ask the Applicant to follow the same methodology for generational stability should as the others southern blot analysis (i.e. using the same probes).
- The regulator is encouraged to ask the Applicant to present molecular size markers when facilitating the analyses.
- The regulator is encouraged to ask the Applicant to use molecular profiling techniques to allow a more thorough study of the insert genetic stability over multiple generations.
- The regulator is encouraged to ask the Applicant to provide the electropherograms in order to check the quality of the sequences.
- The regulator is encouraged to ask the Applicant to conduct generational sequencing studies.

2.2.3 Information on the expression of the insert.

Levels of DMO and PAT were determined in protein extracts from MON88701 seed by ELISA. These seed were analyzed at maturity and both dicamba/glyphosinate treated and untreated seed were analyzed. However, the Applicant does not clarify if the mode of treatment is as it would be performed during agricultural production.

The mean DMO and PAT levels in dicamba and glyphosinate treated MON88701 seems to be slightly higher in the treated seed than in the non-treated seed. Difference for DMO is 17.6% higher for the treated in fresh weight basis samples as compared to the non-treated one. For PAT the difference is 5.2%. The Applicant claims that DMO and PAT levels are comparable between the two and that the application of dicamba and glyphosinate does not alter the expression of DMO and PAT. The difference that is present is not commented on further as to whether this is considered as natural variation or else.

Recommendation:

- The regulator is encouraged to ask the Applicant to clarify if the mode of treatment is as it would be performed during agricultural production.
- The regulator is encouraged to ask the Applicant to comment on the difference in the DMO and PAT expression levels in the treated and non-treated seed

4. Toxicology Assessment.

Toxicological testing of newly expressed proteins

The toxicology assessment was performed by comparing biochemical characteristics as; history of safe use, structural similarity to known toxins or other biologically active proteins, acute toxic effect and rapid digestion in mammalian gastrointestinal systems.

All proteins tested were of bacterial origin, here; produced in *E.coli*. From our point of view, the plant version should be used for such purposes even though the concept of equivalence is proven by structure analysis (sequencing). This means that the protein that actually is expressed in the gene modified species, and derived from it, should be used due to the potential differences that can arise because of post translational differences between species, tissues and stages of development (Gomord et al 2005, Küster et al 2001).

DMO protein:

Molecular weight and purity analysis were performed by SDS-PAGE. The molecular weight standard used is not good for accurate size determination of the protein. Another standard should have been used. Also, double band in MON88701 derived DMO between 66.2 and 97.4 kDa is not commented upon. This double band is not visible in the *E.coli* derived protein; a single band can be seen around the same size here.

The equivalence in immune-reactivity was also analysed by western using polyclonal anti-DMO antibodies. Both plant and bacterial version of DMO was recognized by the antibody. For the bacterially derived protein; the antibody recognized higher levels of a band at 75 kDa at high concentrations of the protein. This is not as apparent for the plant derived protein, where the protein recognized at this size has the same level although the overall protein concentration increases. This difference between the plant and the bacterially derived proteins are not commented upon. This band is not analysed further. It should have been sent for MS analysis to verify whether it was a DMO variant or not.

Glycosylation equivalence was also analysed for the two proteins and no glycosylation pattern was detected by the method used. The membrane for analysis of glycosylation do however lack signals from the molecular weight standards, thus it will be impossible to interpret size of bands (if they were present for the *E.coli* and plant derived proteins).

Functional activities of plant and bacterially derived proteins were also investigated. The activities between the two differs with an approximately 30 % higher functional activity of the bacterial version as compared to the plant version (mean values compared). This is however within the “acceptance criterion” by the applicant.

PAT protein:

Molecular weight and purity analysis were performed with SDS-PAGE between MON88701 and *E.coli* derived PAT protein. Their Mw was found to be equal. However, the molecular weight marker used is not good for this purpose as there should have been a band closer to the expected size of the PAT.

Equivalence in immune-reactivity was also analysed using western blot. The molecular weight marker is totally absent on the membrane (Figure 25), so size determination will be

difficult. However, there are no additional bands on the membrane with this detection method and time. There seem to be no difference in immune-reactivity between plant and microbially derived protein.

Glycosylation pattern/equivalence was also analysed and found to be equal for both PAT proteins. None of the proteins were found to be glycosylated by this method. However, the signals from the positive controls are weak, so the membrane could have had a longer exposure time to see if this resulted in more bands.
Functional activities of both plant and microbially derived PAT protein were analysed and found to fall into the pre-set acceptance criterion set by the applicant. However, the microbially derived protein has 26.9% higher activity than the plant derived PAT (when comparing the means values (table 27).

Interactions between PAT, DMO and other proteins were not expected due to their high substrate specificity and thus not analysed.

Recommendation:
- The regulator is encouraged to ask the Applicant to clarify size determination and bands in the protein analysis and provide pictures that makes it possible to draw conclusions.

4.2.3 Stability of protein under processing and storage conditions
The processing of the cotton seeds will expose the seeds to temperatures between 88-130 degrees (food processing) and up to 230 degrees for the deodorisation of the oil. The PAT and DMO proteins isolated from *E.coli* (not the plant version) were tested for their stability at temperatures up to 95 degrees at 30 minutes. Both proteins were stable up to these temperatures. The PAT protein had 9% relative activity after 30 minutes at 95 degrees, while the DMO protein had less than 22% relative activity already after 30 minutes at 55 degrees.

Both PAT and DMO are rapidly degraded in SGF and SIF. It is the *E.coli* version of the purified protein that is tested.

Also, based on the low human exposure, history of safe use, lack of homology to known toxins, digestion in SGF, deactivation by heat treatment and lack of acute toxicity; no further feeding studies with the plant derived material containing BOTH of the proteins are performed. It must be emphasized that the cotton in question or food derived thereof has not been subjected to a feeding study for toxicology analysis.

Recommendation:
- The regulator is encouraged to ask the Applicant to use the plant version of the protein in these analyses to get the most authentic results.
- The regulator is encouraged to ask the Applicant to consider toxicity study with the two proteins in combination.

Feeding studies

Repeated dose toxicity studies using laboratory animals (p 161)

E. coli produced MON 88701 DMO protein and *E. coli* produced PAT (bar), in independent studies, were administered as a single dose by oral gavage to 10 male and 10 female CD-1 mice at a dose level selected based on the risk assessment principles of hazard identification and margin of exposure. Each study contained an additional control group of 10 male and 10 female mice. The proteins were not administered together as they would be present in the plant.

Clinical signs, body weights, body weight changes, food consumption, and gross necropsy findings were evaluated in this study. In conclusion, there were no adverse effects of the *E. coli*-produced MON 88701 DMO- and PAT protein when administered by oral gavage at a specific dose (mg protein/kg body weight/day) in male and female mice.

Recommendations:

- The regulator is encouraged to ask the Applicant to use the plant version of the protein in these analyses to get the most authentic results.

Oral toxicity study (p 162)

On the background of the results of the repeated dose toxicity studies the Applicant concluded that a 28-day oral toxicity study for the MON 88701 DMO or PAT proteins was not necessary to perform to confer MON88701 safety (p 162).

90-day feeding study in rodents (p. 164)

Based on the weight of evidence through extensive molecular characterization, history of safe use, lack of structural similarities with known protein toxins and allergens, absence of acute toxicity in oral gavage studies in rodents and rapid digestion in simulated gastric fluid, the Applicant claims that data from a 90-day feeding study in rodents is not required to demonstrate the safety on human or animal health of MON 88701

Recommendations:

- The regulator is encouraged to as the Applicant to include long term exposure-/feeding, using herbicide treated cotton in animal experiment, before a GM plant product is released on the marked for food/feed consumption.

5.0 Allergenicity

Assessment of allergenicity was performed according to the guidelines by Codex Alimentarius by looking at the source of the protein, structural similarities to known allergens, portion of the total protein, speed of digestion in mammalian gastrointestinal systems and stability to heat treatment.

The Applicant claims that there is no reason to expect that the use of MON88701 will significantly increase the intake and exposure to cotton. Any overexpression of the protein is therefore not expected.

5.1.1 Amino acid sequence homology

Neither PAT nor DMO has sequence similarities to known allergens.

5.1.2 Specific serum screening

No serum screening is performed to test for allergenicity towards PAT, DMO or the two in combination. This is due to the long history of these proteins background, use, concentration and lack of sequence homologies to allergens etc. However, these proteins have not been tested "as is" in the plant, neither separately nor together.

Recommendations:

- The regulator is encouraged to as the Applicant to perform serum screening for allergenicity purpose.

5.1.3 Digestibility

Both *E.coli* derived PAT and DMO are rapidly degraded in SIF and SGF. This is used as criteria for evaluating if the proteins are allergic or not. However, it is not clear at what pH these digestions are performed. pH will vary in the stomach in individuals, and might influence the degradation of a protein. This has been shown for other proteins (Guimaraes et al 2010).

Western blots used to show the digestibility (SGF and SIF) of PAT and DMO lack visible molecular weight markers. Size interpretation of bands is thus very difficult.

Recommendations:

- The regulator is encouraged to as the Applicant to clarify at what pH the digestion studies are performed.

5.2 Allergenicity of the whole plant

Not performed.

Cotton is normally no considered to be an allergenic plant. It is not discussed whether or not it is likely that pollen from MON88701 cotton would be allergic. However, as the application is not for growth, this would not be an issue.

Missing information in relation to requirements under the Norwegian Gene Technology Act

Social utility and sustainability aspects

In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act. In accordance with the aim of the Norwegian Gene Technology Act, production and use of the GMO shall take place in an ethically and socially justifiable way, under the principle of sustainable development. This is further elaborated in section 10 of the Act (approval), where it is stated that

"*significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development*".

These issues are further detailed in the regulation on consequence assessment section 17 and its annex 4.

The Norwegian Gene Technology Act, with its clauses on societal utility and sustainable development, comes into play with a view also to health and environmental effects in other countries, such as where GMOs are grown. Although the literature concerning the socio-economic aspects related to the cultivation (and to a much lesser extend the use) of GM cotton is extense (for a review, see e.g. Glover, 2010; Smale et al., 2006), the Applicant does not mention any these references, nor there is an attempt to identify how Mon 88701 cotton might contribute to sustainability and social utility (neither in the producing countries nor in Norway or Europe). Therefore, the Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values, social justification of the GMO within a sustainable development. Given this lack of necessary information for such an evaluation, the Applicant has not demonstrated a benefit to the community and a contribution to sustainable development from the use of Mon 88701 cotton.

Further, published reviews on aspects related to societal utility (e.g. impacts among poor, small-scale farmers in developing countries, share of the benefits among sectors of the society) indicate that these effects have been very complex, mixed and dependent on the agronomic, socio-economic and institutional settings where the technology has been introduced (Glover, 2010). It is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, genetic and socio-economic contexts as regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all likely to affect the outcomes. Hence it cannot be expected that the same effects will apply between different environments and across continents.

On the sustainability of the product, Mon 88701 confers cotton tolerance to herbicides containing glufosinate-ammonium and dicamba. Glufosinate-amonium is a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans.

Dicamba is a synthetic auxine considered as an herbicide with low toxicity, but with high residuality. However, a recent article has been published indicating indirect negative effects of dicamba on insects, while highlighing that few research has been conducted on the issue to date in despite dicamba is, along with 2,4-D, causing most herbicide-drift damage to nontarget plants even though present limited agricultural usage (Bohnenblust et al., 2013). In regards to the tolerance to dicamba, although it is expected that this modification could provide an alternative for controlling weeds in glyphosate-tolerant cotton fields and for extending the effective lifetime of glyphosate (Behrens et al., 2007), the effectiveness of dicamba for controlling weeds such as waterhemp is lower than glyphosate, which could cause a "treadmill" effect for farmers if control is low. Besides, reduced sensitivity to dicamba has also been recently reported in *Amaranthus* species by Bernard et al (2012), which makes the authors conclude that "The commercialization of soybean, cotton, and corn resistant to 2,4-D and dicamba should be accompanied by mandatory stewardship practices that will minimize the selection pressure imposed on other waterhemp populations to evolve resistance to the synthetic auxin herbicides". As this application excludes the cultivation of Mon 88701 in the European Unión, in page 217, II Part – Scientific information, the Applicant states that "... an assessment of the impacts of specific cultivation, management and harvesting techniques it is not relevant given the scope of this application". However, the Gene Technology Act applies not only for Norway but also for cultivating countries, and therefore, information for the risk assessment on the cultivation, management and harvesting stages as well as the post market environmental monitoring is required in order to assess the sustainability criteria laid down in the Act.

The Applicant has not provided information on how long (e.g. number of planting seasons) it will take before the Mon 88701 containing plants develop sensitivity to the combined glufosinate-ammonim and dicamba herbicides. Therefore, it would be incongruent with the principle of sustainable development.

The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues. It is also important to evaluate whether alternative options (e.g. the parental non-GM version of this Mon 88701) may achieve the same outcomes in a safer and ethically justified way.

Ethical considerations

The evaluation of coproducts, that is, secondary products that are specifically designed and intended to be used in conjunction with the GMO, is considered important in the risk assessment of a GMO (Dolezel et al, 2009; Graef et al., 2012). Therefore, considerations of the co-products also warrant an evaluation of safe use.

The event contain the bar gene (from Streptomyces hygroscopicus) encoding for a ephosphinothricin acetyl transferase (PAT) that confers tolerance to herbicides containing glufosinate ammonium, a class of herbicides that are banned in Norway. While it is understood that the Applicant has not applied for deliberate release of Mon 88701 in Norway, the acceptance of a product in which the intended use includes the use of a product banned in Norway would violate basic ethical and social utility criteria, as laid out in the Act. That is,

we find that it would be ethically incongruous to support a double standard of safety for Norway on one hand, and safety for countries from which Norway may import its food and feed on the other. This line of reasoning is consistent with the provisions under the Act to assess ethical, social utility and sustain able development criteria not only for Norway, but for countries from which Norway imports food and feed.

Therefore, we find it difficult to arrive at justified use of this event without engaging in such an ethical double standard. Specifically, this issue is relevant particularly in revised regulations of 2005 Section 17 “Other consequences of the production and use of genetically modified organisms” points 2 and 3 “ethical considerations that may arise in connection with the use of the genetically modif ied organism(s), and “any favorable or unfavorable social consequences that may arise from the use of the genetically modified organism(s)”, respectively.

Recommendation:

- The regulator is encouraged to ask the Applicant to submit required information on the social utility of MON 88701 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Conclusion

Available information for risk assessment evaluation

This evaluation is based on the Applicant's own submitted information, along with our own expertise in related fields. The relevant scientific literature provided in the application is very limited in some cases, yet we have tried to extract information from the peer-reviewed literature that may inform the scientific validity of the information under consideration. In situations where lack of knowledge, complexity and uncertainty are high, particularly in relation to unknown adverse effects that may arise as a result of approval for release of a living modified organism into the environment or food supply, the available information may not be sufficient to warrant approval. Further information may address some of these issues, however an accurate description of uncertainties provided by the applicant would provide a more useful basis for assessing the level of risk that may come with regulatory approval of the GMO, taken on a case-by-case basis.

In all cases, product-related safety testing should have an independent and unbiased character. This goes both for the production of data for risk assessment, and for the evaluation of the data.

The lack of compelling or complete scientific information to support the claims of the Applicant documented here highlights the need for independent evaluation of the dossier as performed here, including the raw data produced by the Applicant. We therefore support better transparency and independent review of information to ensure high standards within the regulatory process. This would include any information provided by the Applicant used to justify confidentiality claims on any scientific data. We encourage the authorities to insist on this level of transparency and accessibility to all scientific data (including raw data) to ensure the scientific validity of the information presented.

Overall recommendation

Above we highlight a number of issues in relation to the questionable safe use of **MON 88701** that do not justify a conclusion of safe use, social utility and contribution to sustainable development. Critically, the Applicant's environmental monitoring plan lacks sufficient details and descriptions to support the required monitoring activities, and has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of MON 88701 we conclude that based on the available data, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

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